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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/727,509	10/22/96	DARZYNKIEWICZ Z.	1075-PCT.

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EXAMINER	
REES, D	

ART UNIT	PAPER NUMBER
1807	#5

DATE MAILED: 09/05/97

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/727,509	Applicant(s) Darzynkiewicz et al.
	Examiner Dianne Rees	Group Art Unit 1807

☒ Responsive to communication(s) filed on *10/22/96, 1/27/97* .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-20* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-20* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☒ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *2,4*

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Drawings

1. This application has been filed with informal drawings which are acceptable for examination purposes only. The Drawings have been objected to by the Draftsperson under 37 CFR 1.84 (see attached PTO 948). Formal drawings will be required when the application is allowed.

Information Disclosure Statement

2. Applicants' Information Disclosure Statement filed 1/27/97. The references therein have been considered and a number of typographical errors in Applicant's 1449 have been corrected by the Examiner (Applicant is directed to the attached copy of the 1449). Copies of the US Patents cited were not provided by Applicant, however, these references were obtained by the Examiner and considered.

Specification

3. This application does not contain an abstract of the disclosure as required by 37 CFR 1.72(b). An abstract on a separate sheet is required.

4. The disclosure is objected to because of the following informalities:

In the "Brief Description of the Drawings", Figure 2, shows four separate panels but it is not clear what "results" each of these panels represents..

On page 18, where Collins et al. is cited, it appears that "45 1" should be --451--.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

5. Claims 4,8, 11-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The following phrases render the claims vague and indefinite:

Claim 4 is indefinite in the recitation of an improper Markush group (reciting "selected from the group of"). Therefore it is unclear if applicant intends that the genus is limited to the species recited in the group or might include other species. The claim should be amended to recited --selected from the group consisting of--. See also claim 8, 11,14,15,19,20 for similar language.

Claim 12 is indefinite in the recitation of "the resulting BrDUrd-DNA strands" as the term lacks proper antecedent basis. Further there may not be any resulting BrDUrd-DNA strands if there are no breaks,. The claim might be amended to recite --reacting any resulting BrdURd-DNA strands--. See also claim 17 at step (c).

Claim 12 is also indefinite in lacking a step that completes the preamble of the claim, i.e a step whereby detecting said label is correlated with the presence of breaks in said DNA strands.

Claim 20 is indefinite in the recitation of "light of the excitation wavelength" (see page 26, last line) as "the excitation wavelength" lacks proper antecedent basis

Claim Rejections - 35 U.S.C. § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3,5-7, 9-10, are rejected under 35 U.S.C. 102(b) as being anticipated by Jirkowski et al (Histochemistry 91: 51) .

Jirkowski et al.teaches a method of labelling DNA strands comprising incubating DNA strands (in this example, oligonucleotide probes) with a halogenated deoxynucleotide triphosphate (in this example,a brominated deoxynucleotide triphosphate- 5-bromo-2' deoxyuridine triphosphate) using an enzyme (terminal deoxynucleotidyl transferase) that catalytically attaches the halogenated deoxynucleotide (in this example, a brominated deoxynucleotide- 5'bromo-2'dexyuridine monophosphate) onto the 3' end (i.e the 3'-OH end) of said DNA strand. (See page 51, column 2 "Terminal labelling with 5-BrdU"). The labelled strands were then reacted with a anti-halogenated deoxynucleotide antibody which specifically binds to the halogenated nucleotide (in this example, the antibody is a monoclonal antibody to 5-BrdU)(see page 51, column 2, "In situ hybridization", paragraph 2, line 13)

7. Claims 1-11 are rejected under 35 U.S.C. 102(b) as being anticipated by Vanderlaan et al. (USPAT 5053336, Oct 1, 1991).

Vanderlaan et al teaches a method for labelling DNA strands- in this example, the DNA strands are chromosomal DNA . The method comprises incubating the strands with a halogenated deoxynucleotide triphosphate such as brominated deoxynucleotide triphosphate- 5-bromo-2' deoxyuridine triphosphate and iododeoxyuridine triphosphate using an enzyme (polymerases which are endogenously found within the cell)(see Figures). Replication enzymes inherently catalytically attach deoxynucleotides onto the 3' ends of DNA strands (i.e such as the 3 end of Okasaki fragments that are formed during DNA replication).(Here the 3' end is not interpreted as the terminus of the DNA molecule but as any 3' end exposed on the DNA strand). The resulting HdN -DNA strands are then reacted with a labeled anti-HdN antibody which specifically binds to the HdN, such as a fluoresceinated anti HdN (in this example, an anti-BRdU antibody, see column 13).

Claim Rejections - 35 U.S.C. § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 4, 8, 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jirkowski et al. as applied to claims 1-3,5-7, 9, and 10 above in paragraph 6 and further in view of Vanderlaan et al. (USPAT 5053336, Oct 1, 1991) .

Jirkowski et al. meets all of the limitations of the claims as discussed above except for the teaching of labelled antibodies, such as those recited in claims 4,8, and 11. However, Vanderlaan et al. teaches monoclonal antibodies for the detection of halogenated nucleosides; among those taught are antibodies against BrdU-DNA (see column 12, lines 44-54). Vanderlaan further teaches the conjugation of said antibodies to fluorescent dyes (see columns 13-14) and teaches that antibodies can be coupled to said dyes with full retention of antibody activity .(column 13, lines

35-36). Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time that the invention was made to modify the invention of Jirkowski et al. to include a labelled antibody, such as a fluorescently labelled antibody , given the teachings of Vanderlaan of fluorescent antibodies which retain their specificity and are easily detected, for the expected benefit of avoiding the use of secondary antibodies, thereby saving both reagents and steps.

9. Claims 12-15, 17-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gorcyzca et al. (Cancer Research 53: 1945-1952, April 15, 1993) and further in view of Vanderlaan et al. (USPAT 5053336, Oct 1, 1991) .

Gorcyzca et al. teaches the detection of DNA strand breaks in apoptotic cells by in situ terminal deoxynucleotidyl transferase and nick translation assays. The method comprises labelling fixed cells with biotinylated dUTP or using TdT or nick translation type assays, such that the ends of DNA strands at the sites of DNA breaks, such as incurred during apoptosis, are labelled at their 3'-OH termini with biotinylated dUTP and in turn labelled with fluoresceinated avidin (see abstract). The label is then detected by flow cytometry analysis of cells and apoptosis is confirmed by the detection of label at significantly higher levels than the mean level found in control cells(compare Panel B vs panel A, in Figure 4, for example). Gorcyzca does not teach the use of

halogenated deoxynucleotide triphosphates as the label. However, Vanderlaan et al. teaches that halogenated deoxynucleotide triphosphates are a substrate for polymerases during DNA replication(see column 5,for example, lines 1-26) and may be sensitively detected using fluorescently labeled antibodies against halogenated nucleosides incorporated into DNA strands by using flow cytometry assays. It therefore would have been prima facie obvious to one of ordinary skill in the art at the time that the invention was made, to modify the teachings of Gorcyza et al.to use a halogenated deoxyribonucleotide triphosphate rather than biotinylated dUTP since nick translation is essentially a replication reaction involving the addition of nucleotides with a polymerase at the site of nicks or breaks while replication involves the addition of nucleotides with a polymerase at the site of primers or, in the case of repair synthesis, also at the site of nicks or breaks in DNA strands. Thus one of ordinary skill in the art at the time that the invention was made would expect that halogenated deoxynucleotide triphosphates would perform the same function as biotinylated dUTP and one would be motivated to use the halogenated deoxynucleotide triphosphates of Vanderlaan to increase the versatility of the method. Although not explicitly setting a level for determining apoptosis as being at least two standard deviations above the mean, it would have been prima facie obvious to one of ordinary skill in the art at the time that the invention was made to set the level of standard deviation as at least one since anything less would represent normal variation (i.e about two); one of ordinary skill in the art at the time that the invention was made would be motivated to set the standard deviation as being two to determine significance to increase the reliability of one's results.

10. Claim 16 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gorcyzca et al. (Cancer Research 53: 1945-1952, April 15, 1993) in view of Vanderlaan et al. as applied to claims 12-15, 17-20 and further in view of Keydar et al. (USPAT 470738, Nov 17, 1987)

Gorrcyza et al. in view of Vanderlaan et al. meet all of the limitations of the claim as discussed above except for the teaching of radiolabelled antoibodies. However, Keydar et al. teaches a variety of means may be used to label antibodies , uncliding radioactivity and that antibodies labelled in this way may be readily detected by scintillation counting, radiography, or other methods that can detect radioactive decay (i.e for example, Geiger counters)(see column 4, lines67). Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time that the invnetion was made to modify the method of Gorcyza et al. in view of Vanderlaan to use an anti HdN antibody which is radioactively labelled given the teachings of Keydar that said antibodies may be readilty detected and quantitated (see column 14) and that these would function equivalently as the fluorescently labelled antibodies of Vanderlaan.

It is noted that a rejection is not applied over Gorcyza et al. in view of Jirkowski et al. (and further in view Keydar) despite the fact that Jirkowski et al. teaches that a halogenated

deoxynucleotide triphosphate such as BrdU is a substrate for terminal nucleotidyl transferase.Although one of skill in the art at the time that the invention was made might have expected that BrdU would be added as efficiently to a DNA strand breaks, such as induced during apoptosis, by TdT, Applicants' have demonstrated unexpected results with regards to the sensitivity of the method in labelling of such breaks in comparison to prior art methods (See Figure 1 and Applicant's specification at pages 14-15). However, Applicant have not demonstrated such results for the incorporation of halogenated deoxynucleotides which are added to DNA breaks by polymerases, thus there is no evidence on the record to rebut the Examiner's prima facie case that halogenated deoxynucleotides would function equivalently as biotinylated dUTP when added to a 3'OH end of a DNA molecule at a DNA break by a polymerase.

The rejections of claims 12-20 might be overcome by limiting the claims to the use of TdT or alternatively to provide evidence that similar unexpected results are obtained when halogenated deoxynucleotides are added by polymerases.

No claims are allowed.

Papers related to this application may be submitted to Group 1800 by facsimile transmission via the P.T.O. Fax Center located in Crystal Mall 1. The CM1 Fax Center numbers for Official Communications are (703) 305-3014 and (703) 305-4227. Please note that the faxing of such papers must conform with the notice to Comply published in the Official Gazette, 1096 OG 30 (Nov 15, 1989). Applicant is informed that all Official communications that go through the Fax

Center will not be forwarded directly to the Examiner but will be routed through docketing. Applicant is encouraged to clearly mark any communications to the Office as DRAFT, OFFICIAL (and further as RESPONSE TO OFFICE ACTION, or AFTER FINAL. etc.) For any inquiries concerning the status of of a Faxed Communication please contact (703) 308-9378.

An inquiry regarding the Office Action should be directed to examiner Dianne Rees, Ph.D., whose telephone number is (703)308-6565.. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones, can be reached on (703) 308-1152.

Calls of a general nature may be directed to the Group receptionist who may be reached at (703) 308-0196.

Dianne Rees
8/28/97

DIANNE REES
PATENT EXAMINER
ART UNIT 1807
8/28/97